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(71) Applicant (*for all designated States except US*): DANBIOSYST UK LIMITED [GB/GB]; Albert Einstein Centre, Highfields Science Park, Nottingham NG7 2TN (GB).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): ILLUM, Lisbeth [DK/GB]; 19 Cavendish Crescent North, The Park, Nottingham NG7 1BA (GB). WATTS, Peter, James [GB/GB]; Flat 2, 47 Highfield Road, West Bridgford, Nottingham NG2 6DR (GB).

(74) Agent: BASSETT, Richard; Eric Potter Clarkson, St. Mary's Court, St. Mary's Gate, Nottingham NG1 1LE (GB).

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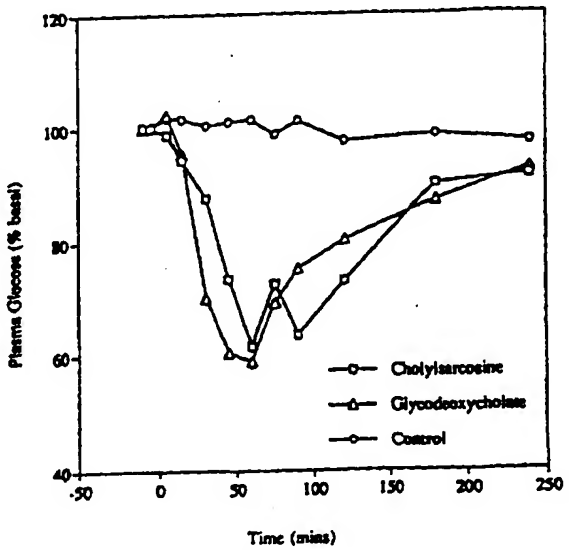
(57) Abstract

A composition for administration to a mucosal surface of a mammal comprising a non-metabolisable bile salt analogue and a therapeutic agent. Preferably the non-metabolisable bile salt analogue is a non-naturally occurring conjugate of cholic acid and an amino acid, and in particular cholylsarcosine. Preferably the therapeutic agent is a polar molecule.

Plasma Glucose Concentration after Colonic Dosing to Pigs



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COMPOSITION FOR ENHANCED UPTAKE OF POLAR DRUGS FROM MUCOSAL SURFACES

This invention relates to novel compositions for enhancing the absorption of polar molecules from the mucosal surfaces of a mammal, in particular the proximal colonic region of the intestine of said mammal.

Background

Administration of drugs to the gastrointestinal tract is a preferred method for both human and veterinary medicine. Most conventional drugs are well absorbed from the intestines, usually by a process of passive diffusion, although certain compounds are taken up by more specific mechanisms such as facilitated or active transport. Polar molecules such as cimetidine, ranitidine, sodium cromoglycate, bisphosphonates (such as clodronate) and captopril often display poor or erratic absorption when administered orally. Polypeptide and polysaccharide drugs, such as insulin, calcitonin, parathyroid hormone or fractions or analogues thereof, luteinising hormone releasing hormone (LHRH or analogues thereof, (eg. nafarelin, buserelin and goserelin)), growth hormone, growth hormone releasing hormones, colony stimulating factors, erythropoietin, somatostatin, interferons and heparins, cannot be given orally because, not only are they poorly absorbed because of their polar nature and size, but they can also be degraded by the endogenous enzymes present in the gastrointestinal tract. If such drugs are given orally, the absolute bioavailability (i.e. the quantity reaching systemic circulation) is generally low when compared to parenteral administration (e.g. less than 1%). Cyclosporin, a polypeptide, is a notable exception to this rule. It is non-polar in nature and has a partition coefficient (octanol/water) of more than 1000. This drug is quite well absorbed from the gastrointestinal tract.

Various attempts have been made to solve the inherent problems connected with the administration of polar drugs, including attempts to improve transmucosal and indeed oral absorption. Strategies which have been employed include chemical modification, undertaken with a view to stabilising the drug and/or making it more lipid soluble, and thus improving the likelihood of diffusion across the lipid membrane or the gastrointestinal tract. Other workers have added stabilising agents, such as peptidase inhibitors (e.g. aprotinin) to reduce metabolic loss, whilst others have used various absorption promoting agents, in the form of non-ionic surface active agents, natural bile salts and analogues thereof (such as sodium taurodihydrofusidate), phospholipids, chelating agents and acyl carnitine in order to improve transmucosal absorption.

Such previous attempts have been well reviewed in the relevant literature. For example, the various attempts to enhance the intestinal permeability of proteins, peptides and other polar drugs have been reviewed by Swenson and Curatolo (Advan. Drug Del. Rev. 8, 39 (1992)). Enhancers which have been employed include the natural bile salts and their mixtures with other materials such as oleic acid. For example, the ileocolonic delivery of insulin at 10 units/kg to the dog using a mixed micelle system comprising sodium glycocholate (30 mM) and a fatty acid (linoleic acid) at 40 mM has been described by Scott-Moncrieff and others (J. Pharm. Sci. 83, 1465 (1994)). The reported bioavailability was 1.4%.

Although bile salts and their derivatives and analogues (e.g. sodium taurodihydrofusidate) are known to be good enhancing agents, not only when used in the gastrointestinal tract, but also when used in conjunction with other mucosal surfaces (such as those found in the nose, genitourinary tract, lung and buccal cavity), the problem exists that these materials cannot normally be used in man because of their irritant nature.

Conjugated bile salts have also been employed within the gastrointestinal tract. However, such materials are metabolised, leading to the problem that they possess poor absorption enhancing ability. Unconjugated bile salts are also known to cause problems within the intestine, particularly in the large bowel, because of their secretory effects, which can give rise to gastrointestinal disturbances and diarrhoea. Indeed, in the past, unconjugated bile salts have been used as laxative agents.

Therefore, there remains a need for absorption enhancers for use in the administration of drugs which are poorly absorbed from mucosal surfaces, which may be readily administered to the mucosal surfaces, which are effective at a low dose, which are minimally damaging to cells, and which have no effect on the normal physiology of the site of administration. When the site of administration is the intestine, this last requirement refers specifically to water secretion and the induction of diarrhoea.

We have found, surprisingly, that the above problems may be solved by the administration of compositions including therapeutic agents and non-metabolisable bile salt analogues, including the synthetic bile salt derivative cholylsarcosine. We have found that non-metabolisable bile salt analogues, including cholylsarcosine, improve the absorption of poorly absorbed polar molecules across mucosal surfaces such as the gastrointestinal tract, the nose, the vagina, the buccal cavity and the rectum.

Thus, according to the first aspect of the invention there is provided a composition for administration to a mucosal surface of a mammal comprising a bile salt analogue and a therapeutic agent, characterised in that the bile salt analogue is non-metabolisable, hereinafter referred to as "the compositions according to the invention".

By "non-metabolisable" we mean a compound which is not capable of being metabolised under normal conditions experienced within the gastrointestinal tract, for example a compound which cannot be hydrolysed below 40°C at pHs within the range 5 to 8. Compounds which may be employed in the compositions according to the invention include cholylsarcosine, and other non-naturally occurring, non-metabolisable, conjugates of cholic acid and amino acids. Preferred compounds which may be employed include cholylsarcosine.

Cholylsarcosine is a conjugated bile acid analogue that has been developed as a bile acid replacement agent. It is a synthetic conjugate of cholic acid and sarcosine.

In humans, it is not metabolised following administration and is non-toxic (Schmassmann *et al.*, Gastroenterology 104, 1171-1181, (1993)). When infused into the colon of rabbit, cholylsarcosine did not influence water absorption or permeability to erythritol in comparison to chenodeoxycholate which induced vigorous water secretion. There was little absorption of cholylsarcosine from the colon. US 5079240 discloses that cholylsarcosine is useful in bile acid replacement therapy, in particular, in view of the fact that is possesses resistance again bacterial deconjugation. However, the use of cholylsarcosine as an absorption promoting agent has not been described previously.

Although the compositions according to the invention may be used with therapeutic agents which are of a non-polar or a polar nature, we prefer that the therapeutic agent is a polar compound or polar molecule.

A polar compound or polar molecule is defined herein as a compound with a partition coefficient between water and octanol at pH 7.4 of less than 10.

The polar compound may possess a molecular weight from 100 Da to 100,000 Da. A preferred range is 300 Da to 30,000 Da.

Although it is not an essential requirement, it is preferred that the polar molecule is uncharged. The following list of polar compounds, which are suitable for use in the compositions according to the invention, is provided by way of illustration and is not meant to be exclusive: cimetidine, ranitidine, sodium cromoglycate, bisphosphonates such as clodronate; angiotensin converting enzyme (ACE) inhibitors such as captopril and sampatrilat; polypeptide and protein-based drugs such as insulin, calcitonins, parathyroid hormone, fractions thereof or analogues thereof, luteinising hormone releasing hormones or analogues thereof such as nafarelin, buserelin, goserelin, growth hormone, growth hormone releasing factors or hormones, parathyroid hormone and parathyroid related hormones, colony stimulating factors, erythropoietin, somatostatin, α-, β- or γ-interferon, proinsulin, glucagon, vasopressin, desmopressin, thyroid stimulating hormone, atrial peptides, tissue plasminogen activator, factor VIII, cholecystokinin, octreotide; polysaccharide drugs or glycosaminylglycols, such as low molecular weight heparin; antisense agents such as an oligonucleotide; and DNA or a therapeutic gene for gene therapy, such as DNA vaccines.

Combinations of any of the above therapeutic agents may be used.

Preferred therapeutic agents which may be used in the compositions according to the invention include insulin, calcitonin, captopril, growth hormone, heparins, bisphosphonates, desmopressin, colony stimulating factors, α-interferon, γ-interferon, erythropoietin, parathyroid hormone and parathyroid related hormones.

The compositions according to the invention may be administered orally, nasally, vaginally, buccally or rectally in a variety of pharmaceutically acceptable dosing forms, which will be familiar to those skilled in the art. For example, compositions for nasal administration may be administered as a solution *via* a nasal spray or as a powder *via* a nasal insufflator; compositions for buccal administration may be administered *via* buccal patches or buccal tablets; compositions for vaginal administration may be administered as gels or in the form of vaginal suppositories (pessaries); and compositions for rectal administration may be administered as suppositories. However, we prefer that the compositions according to the invention are administered orally, in the form of a tablet, a capsule or a pellet or a microsphere system, all of which may be formulated in accordance with techniques which are well known to those skilled in the art.

According to a further aspect of the invention there is provided a process for the preparation of a composition according to the invention, which process comprises mixing together a non-metabolisable bile salt analogue and a therapeutic agent in a pharmaceutically acceptable dosing form.

Compositions according to the invention which may be administered orally may be adapted to deliver therapeutic agent to the small intestine or the colonic, especially the proximal colonic, region of the gastrointestinal tract. Preferably, a means is provided to prevent release of therapeutic agent until the formulation reaches the small intestine or colon.

Means which may be employed in order to prevent release until the small intestine is reached are well known to those skilled in the art. Suitable systems include dosage forms coated with so-called enteric polymers that do not dissolve in the acidic conditions which exist in the stomach, but

dissolve in the more alkaline conditions found in the small intestine of a mammal. Suitable enteric coating materials include modified cellulose polymers and acrylic polymers, and in particular those sold under the trademark Eudragit®.

For colonic delivery, a coated capsule system can be employed as described in international patent application No. PCT/GB94/12394, which provides a drug delivery composition for delivering a drug to the colonic region comprising a starch capsule containing the drug and wherein the starch capsule is provided with a coating such that the drug is predominantly released from the capsule in the colon and/or the terminal ileum. Other colonic delivery systems which may be employed include those described in international patent application No. PCT/GB96/01933.

Further, site specific delivery to the colon may also be achieved by using capsules or tablets that are coated in materials which are specifically degraded in the colonic environment by the action of microorganisms and/or the reductive environment found there. Such materials include, but are not limited, to azo polymers and disulphide polymers (see, for example, international patent application No. PCT/BE91/00006), amylose (see, Milojevic *et al*, Proc. Int. Symp. Contr. Rel. Bioact. Mater., **20**, 288 (1993); Allwood *et al*, international patent application No. PCT/GB90/25373), calcium pectinate (see Rubenstein *et al*, Pharm. Res., **10**, 258 (1993)), pectin (see Pharm. Res. **12**, suppl. S-221 (1995)), chondroitin sulphate (see Rubenstein *et al*, Pharm. Res., **9**, 276 (1992)), modified guar gum (see Rubenstein and Gliko-Kabir, S.T.P. Pharma Sciences, 5, 36-40, 1995), p-cyclodextrin (Siefke *et al*, Eu. J. Pharm. Biopharm., **40**, (Suppl.), 33S (1994)), saccharide containing polymer (Sintov and Rubenstein, international patent application No. PCT/US91/03014), methacrylate-galactomannan (Lehmann and Dreher,

Proceed. Int. Symp. Contr. Rel. Bioact. Mater 18, 331, 1991), dextran hydrogels (Hovgaard and Brøndsted, 3rd Eur. Symp. Control. Drug Del., Abstract book (1994) 87), pH-sensitive hydrogels (Kopecek *et al*, J. Control. Rel., 19, 121, 1992) and resistant starches, e.g. glassy amylose, that are not broken down by the enzymes in the upper gastrointestinal tract but are degraded by enzymes in the colon.

The compositions according to the invention may also be delivered to the colon using colon targeting systems including, but not limited to, the following systems: The Pulsincap™ System (WO 90/09168), which is an oral pulsatile delivery system, and may be configured to release its drug content at a predetermined time or place within the gastrointestinal tract. The device essentially consists of an impermeable capsule body which contains the drug, sealed at the neck orifice with a hydrogel plug. A normal gelatin cap is then placed onto the body of the device. After ingestion, the gelatin cap dissolves allowing the plug to hydrate. At a predetermined, and controlled, time the swollen plug is ejected from the body of the device, thereby releasing the capsule contents and enabling the drug to be released. The Time Clock Release System™ (Pozzi *et al*, APV Course on Pulsatile Drug Delivery, Konigswinter, May 20, 1992), which is a tablet system in which a tablet core containing the active drug is coated with a layer of pharmaceutical excipients. The excipients hydrate causing the surface layer to burst at a set time. Another system which may be used is the Time Controlled Explosion System, as described in US 4871549 (incorporated herein by reference).

It will be well understood by those skilled in the art that further excipients may be employed in the compositions according to the invention. For example, in solid dosing forms, further excipients which may be employed include diluents such as microcrystalline cellulose (e.g. Avicel®, FMC),

lactose, dicalcium phosphate and starch(es); disintegrants such as microcrystalline cellulose, starch(es) and cross-linked carboxymethylcellulose; lubricants such as magnesium stearate and stearic acid; granulating agents such as povidone; and release modifiers such as hydroxypropyl methylcellulose and hydroxypropyl cellulose. Suitable quantities of such excipients will depend upon the identity of the active ingredient(s) and particular dosing form which is used.

Appropriate quantities of non-metabolisable bile salt analogues which may be employed in the compositions according to the invention will depend upon the mode of delivery which is employed. The quantity of active ingredient in the formulation may be selected non-inventively by those skilled in the art in order to provide a concentration of between 0.5 and 1% at the appropriate mucosal surface. Thus, in a single unit oral dosage form weighing between 100 mg and 1.5 g, this will mean a quantity of between 10 and 90% w/w. In a solution-based delivery form suitable for nasal delivery, appropriate concentrations will be in the range 0.05 to 30% w/v of non-metabolisable bile salt analogue in the composition.

Compositions according to the invention have been found to have the advantage that they may be readily administered to the mucosal surfaces, are effective at a low dose, are minimally damaging to cells, and have no adverse effect on the normal physiology of the administered site. In particular, the compositions according to the invention have the advantage that they permit the oral administration of drugs that are poorly absorbed from the gastrointestinal tract, or cannot be given orally by means of known techniques because of low bioavailability.

According to a further aspect of the invention there is thus provided a method for the improved administration (in particular oral administration)

of therapeutic agents (which, in particular, are poorly absorbed *via* mucosal surfaces (in particular the gastrointestinal tract)), which comprises administering a composition according to the invention to a patient, preferably a human patient.

The amount of therapeutic agent which may be employed in the compositions according to the invention will depend upon the agent which is used. However, it will be clear to the skilled person that doses of therapeutic agents can be readily determined non-inventively. For example estimates of dosage can be made from known injectable products assuming that 10% of the dose is absorbed.

Some suitable doses for selected drugs in the present invention are as follows:

Insulin	20 i.u./kg
Calcitonin	100 units/kg
Captopril	100 mg
Growth hormone	10 units/kg
Heparin	10,000 units
Bisphosphonates	200 mg
Desmopressin	100-300 p,g
Colon stimulating factors	3 million units/kg
Interferon α	10 million units
Interferon γ	18 million units
Erythropioetin	200 units/kg

The invention is illustrated, but in no way limited, by the following examples. The known enhancer material glycodeoxycholate was used as a positive control. A formulation comprising insulin with no enhancer was

used as a further control.

Brief Description of the Figures

Figure 1 shows the plasma glucose concentration following colonic administration to pigs of: (a) insulin in conjunction with the non-metabolisable bile salt analogue, cholylsarcosine; (b) insulin in conjunction with the deconjugated bile salt, glycodeoxycholic acid; and (c) control (no bile salt or analogue thereof).

Figure 2 shows the plasma glucose concentration following nasal administration to sheep of: (a) insulin in conjunction with the non-metabolisable bile salt analogue, cholylsarcosine; (b) insulin in conjunction with the deconjugated bile salt, glycodeoxycholic acid; and (c) control (no bile salt or analogue thereof).

Example 1

Oral absorption of insulin

Formulations containing insulin together with added cholylsarcosine were evaluated in a pig model.

Sodium insulin (Proinsulin derived, Item code QD339G, Lot number 181 EM7, 28.1 IU/mg of pure insulin) was obtained from Eli Lilly and Company, Indianapolis, USA. The purity of the material, previously determined by spectrophotometry, was 88%. This is equivalent to 24.728 IU insulin/mg.

The other chemicals used were as follows: Glycodeoxycholic acid was obtained from Sigma, Poole, Dorset. Avicel (microcrystalline cellulose),

used as a dispersing agent, was obtained from Honeywill & Stein, Sutton, UK. Other dispersing agents which may be used include lactose and silica.

Cholylsarcosine was prepared according to the methods of J. Lillienau, C.D. *et al.* J. Clin. Invest. 1992, 420-431 and Tserng *et al.* J. Lipid Res., (1977) 18, 404, wherein the cholylsarcosine ethyl ester is first produced. A suspension of sarcosine ethyl ester hydrochloride (18.67 g; 122 mmol) in ethyl acetate (HPLC grade) containing triethylamine (17.3 mL, 125 mmol) was stirred at room temperature for 30 minutes. To this solution were added cholic acid (35.5 g; 87 mmol) and N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ; 30 g; 122 mmol) and the mixture was refluxed overnight. Moisture was excluded with the aid of a drying tube. After cooling, the reaction mixture was decanted from the solid residue, washed with 0.5M $NaOH_{(aq)}$, 0.5M $HCl_{(aq)}$, water and brine, dried ($MgSO_4$) and evaporated to yield the title ester as a viscous oil (46 g) (Th. = 44.1 g).

To produce cholylsarcosine the following was undertaken: A solution of cholylsarcosine ethyl ester (44.1 g; 87 mmol; obtained as described above) in ethanol (AR; 175 ml) was heated under reflux and a solution of K_2CO_3 (10%,; 175 ml) was added over 15 minutes. The solution was refluxed for a further 15 minutes, reduced to half its original volume *in vacuo*, diluted with water (300 ml), acidified with 2M $HCl_{(aq)}$ and extracted with ethyl acetate. The pooled organic extracts were washed with water and brine, dried ($MgSO_4$) and evaporated to yield cholylsarcosine as an off-white amorphous solid (25 g).

The free acid prepared above did not easily dissolve in water or aqueous sodium hydroxide solution, but could be readily dissolved in aqueous sodium bicarbonate solution. For biological evaluation in aqueous systems,

the freely water soluble sodium salt was prepared.

A solution of sodium hydroxide (2.2 g, 55 mmol) in methanol (40 ml) was added to a solution of cholylsarcosine (25 g; 49 mmol) in methanol. The product was precipitated from solution with ether (sodium dried works best), wasted with ethyl acetate. After the removal of volatiles *in vacuo* the product was obtained as a white amorphous solid (20.5 g).

For the preparation of each formulation for biological evaluation, the components were weighed into plastic weighing boats, transferred to a glass bottle and mixed thoroughly by gentle shaking and then filled into a starch capsule (Capill™; obtained from Capsugel, Switzerland). The composition of the capsule blend was as follows, so as to provide a fill weight of about 200-220 mg:

Formulation 1	insulin	19.8 IU/kg
	cholylsarcosine	180 mg
	Avicel®	14 mg
Formulation 2	insulin	19.8 IU/kg
	glycodeoxycholic acid	180 mg
	Avicel®	14 mg
Formulation 3	insulin	19.8 IU/kg
	Avicel®	172 mg

The powder blends were then stored desiccated at 4°C until required.

The capsules were then administered to pigs. The pigs had been surgically modified to insert a fistula into the terminal ileum, just above the ileo-

caecal valve, and cannulated at the cephalic vein to allow repeated blood sampling. Three pigs *per* group were used for the study, the mean weight ranged from 30-50 kg. One capsule was administered to each pig, *via* the ileal fistula. Frequent blood samples were taken, plasma separated and analysed for glucose content. The insulin levels in the plasma were measured by a standard radioimmune assay.

The results are shown in Tables 1 and 2 and Figure 1.

It can be seen that for the control formulation (Formulation 3), which contained no added enhancer, there was no measured change to the plasma glucose level. In contrast, for the glycodeoxycholate formulation and the cholylsarcosine formulation, the blood glucose level fell and reached a nadir at about 60 minutes. The reduction was similar for both materials.

Table 2 shows that the reduction in plasma glucose is accompanied by a corresponding rise in plasma insulin levels. The maximum level was 23 mn/l and a measured AUC of 599 mn/l.min.

Surprisingly, the synthetic non-metabolisable bile salt derivative gives the same absorption enhancing effect as does a positive control in the form of a deconjugated bile salt, glycodeoxycholic acid.

TABLE 1
PLASMA GLUCOSE CONCENTRATIONS AFTER THE ILEAL ADMINISTRATION OF INSULIN WITH ENHANCER SYSTEMS IN SOLID FORMULATIONS IN PIGS.

Dose of Insulin 19.8 IU/kg

Time before or after dosing (min)	Plasma glucose (% of basal concentration) Cholylsarcosine		Glycodeoxycholate		No-enhancer	
	Mean	Std Dev.	Mean	Std Dev	Mean	Std Dev.
-10	100.0	0.0	100.0	0.0	100.0	0.0
-5	100.0	0.0	100.0	0.0	100.0	0.0
5	98.7	3.8	102.3	3.1	101.6	4.6
15	94.4	7.0	95.4	15.3	101.5	3.2
30	87.6	11.1	70.1	6.2	100.3	2.3
45	73.4	26.3	60.4	22.2	100.9	2.4
60	41.5	30.3	58.9	23.9	101.3	6.1
75	72.5	20.8	69.1	25.5	98.6	7.2
90	63.4	24.6	75.2	26.6	101.0	8.9
120	73.0	25.9	80.3	8.9	97.4	9.0
180	89.9	11.3	87.1	3.8	93.6	5.7
240	91.6	4.2	92.8	4.8	97.8	5.9

TABLE 2
PLASMA INSULIN CONCENTRATIONS AFTER THE ILEAL ADMINISTRATION OF INSULIN AND POTENTIAL ENHANCERS IN SOLID FORMULATIONS IN PIGS (WITH CHOLYSARCOSINE AS ENHANCER)

Dose of Insulin 19.8 IU/kg

Time before or after dosing (min)	Insulin concentrations (mM) Pig 42	Pig 43	Pig 44	Mean	StdDev
-10	12.1	8.5	2.7	7.8	4.7
-5	12.2	11.2	5.8	9.7	3.4
5	12.0	10.1	2.7	8.3	4.9
15	16.7	12.0	3.3	10.7	6.8
30	19.2	22.9	11.7	17.9	5.7
45	16.3	41.4	16.1	24.6	14.6
60	16.3	43.5	11.6	23.0	17.2
75	19.1	48.8	13.0	27.0	19.1
90	16.4	65.8	3.9	28.7	32.7
120	12.0	21.6	6.2	13.3	7.9
180	12.6	5.2	6.4	8.1	4.0
240	12.0	6.5	4.4	7.6	3.9

Example 2

Oral absorption of salmon calcitonin

A formulation of salmon calcitonin was prepared by mixing the drug with mannitol. The dose per capsule was 75 iu/kg. The cholylsarcosine was prepared as in Example 1 and was added at a concentration of 1.0 mg/kg. The mixture was filled into starch capsules (Capill™ available from Capsugel, Switzerland) and one capsule administered to each of five pigs. Each capsule was administered directly into the terminal ileum *via* a ileal fistula. Blood samples were collected *via* a venous access port. The blood was collected into 4 ml heparinized tubes, at 15 minutes prior to dosing and at 5, 15, 30, 45, 60, 75, 90, 120, 180, 240 and 300 minutes after dosing. Plasma was separated by centrifugation and the pharmacodynamic effect of the drug measured by the hypocalcemic response using a standard procedure. The mean calcium level in the plasma was reduced to reach a nadir of 86% of the basal level at 90 minutes. A control experiment was also performed wherein a starch capsule was filled with salmon calcitonin at a dose of 500 iu/kg (4.09 mg per dose). These control capsules were given to a group of four pigs as before and the blood samples collected as previously. The plasma calcium levels were determined using a standard procedure. Despite the high dose of salmon calcitonin (500 IU/kg) the control formulation failed to demonstrate a reduction in plasma calcium levels even when used at a level more than six times that in the test formulation with cholylsarcosine. The results are shown in Table 3.

TABLE 3
PLASMA CALCIUM CONCENTRATIONS FOLLOWING THE ILEAL ADMINISTRATION OF SALMON CALCITONIN (S-CT) IN A CAPSULE FORMULATION CONTAINING S-CT WITH AND WITHOUT CHOLYLSARCOSINE

Dose of S-CT: 75 IU/kg, cholylsarcosine (1.0 mg/kg)

Time before or after dosing (min)	Plasma calcium (% of basal concentration) Mean	Std. Dev.
-15	100.0	0.0
5	97.5	1.2
15	97.4	0.2
30	94.1	5.0
45	90.7	4.9
60	87.5	5.2
75	85.7	6.4
90	86.1	7.4
120	87.4	8.2
150	88.9	9.6
180	90.5	7.5
240	95.8	0.5
300	95.7	3.2

Dose of S-CT : 500 IU/kg No enhancer

Time before or after dosing (min)	Plasma calcium (% of basal concentration) Mean	Std. Dev.
-10	100.0	0.0
-5	100.0	0.0
5	99.1	4.1
15	99.3	1.8
30	99.8	2.3
45	98.1	1.6
60	97.8	3.2
75	99.6	1.7
90	99.2	3.7
120	97.8	2.5
180	97.1	2.7
240	98.9	3.0
300	97.4	2.4
360	98.6	4.3
480	97.2	7.9
1440	101.4	3.1

Example 3

Nasal absorption of insulin

The nasal absorption of insulin as a simple solution or in combination with cholylsarcosine (0.5%) or glycodeoxycholate (0.5%) was investigated in the sheep model.

Insulin stock solution:

Insulin (161.76 mg; QD339G) was weighed into a 10 ml volumetric flask. The volume was made up to 10 ml with 14.65 mM phosphate buffer and the pH was checked.

Nasal formulations:

For each of the bile salt formulations, 25 mg of the bile salt required was dissolved in 2 ml of 14.65 mM phosphate buffer in a 5 ml volumetric flask. To this was added 2.5 ml of 400 IU/ml insulin stock solution. The contents of each flask were made up to 5 ml with 14.65 mM phosphate buffer.

For the insulin solution formulation without bile salts, 2.5 ml of 400 IU/ml insulin stock solution was measured into a 5 ml volumetric flask. The contents of the flask were made up to 5 ml with 14.65 mM phosphate buffer.

Twelve female, cross-bred sheep of known weight were used in this study. The average weight of the sheep was 49.8 $\pm$ 4.4 kg. The sheep were normally housed outside, but were brought inside for the duration of the study. The animals were not fasted prior to insulin administration. An in-dwelling Viggo secalon cannula was placed approximately 15 cm into one of the external jugular veins of each animal on the first day of the study

and, whenever necessary, was kept patent by flushing it with heparinised (25 IU/ml) 0.9% saline solution.

The sheep were divided into three groups of four animals and each animal received one of the formulations.

The sheep were sedated with an intravenous dose of ketamine hydrochloride at 2.25 mg/kg. This was intended for animal restraint and also as a counter-measure against the animal sneezing during administration. The anaesthesia lasted for approximately 3 minutes.

For intranasal administration of the solution formulation, a blueline umbilical cannula was inserted into the nostril of the sheep before delivery of the appropriate volume of solution (0.01 ml/kg).

Blood samples of 4.0 ml were collected from the cannulated jugular vein of the sheep at 20, 15 and 5 minutes prior to insulin administration and at 5, 10, 15, 20, 30, 40, 60, 75, 90, 120, 150, 180 and 240 minutes post-administration. Blood samples were mixed gently in 4 ml heparinised tubes (Lithium Heparin, 60 IU, Sarstedt, Leicester, U.K.), which were kept on crushed ice before plasma separation. The plasma was separated by centrifugation at 4°C and approximately 3200 rpm. Each plasma sample was divided into two aliquots, one for insulin analysis, and one for glucose analysis. The plasma was then stored at -20°C awaiting insulin and glucose analysis.

Plasma glucose concentrations were analysed using the ERIS 6170 selective multitest analyser from Eppendorf (Olympus). The plasma glucose concentrations (percentage of basal value) are shown in Table 4 and Figure 2.

TABLE 4
PLASMA GLUCOSE CONCENTRATIONS (PERCENTAGE OF THE BASAL VALUE) AFTER THE INTRANASAL ADMINISTRATION OF INSULIN IN VARIOUS SOLUTION FORMULATIONS IN SHEEP

Time before or after dosing (min)	Mean plasma glucose (% of basal concentration)					
	Cholylsarcosine Formulation 1	SD	GDC Formulation 2	SD	Control Formulation 3	SD
-15	100.0	0.0	100.0	0.0	100.0	0.0
-5	100.0	0.0	100.0	0.0	100.0	0.0
5	96.4	1.4	95.3	2.2	98.0	2.6
10	92.4	5.6	92.9	3.9	98.6	2.5
15	84.6	7.9	81.0	5.1	99.2	2.8
20	75.2	8.0	73.8	6.0	98.2	3.8
30	60.4	9.0	59.0	5.6	99.8	2.1
40	58.4	6.1	58.2	5.7	96.4	3.2
50	60.7	12.4	65.8	8.3	96.4	3.2
60	67.8	8.8	75.2	8.1	98.8	4.7
75	78.7	6.2	79.9	5.1	100.5	6.0
90	85.3	6.0	88.2	5.2	98.6	7.3
120	88.5	5.0	89.9	1.7	97.0	6.7
150	93.1	3.0	94.2	2.3	97.0	6.4
180	91.4	3.8	96.0	2.6	99.2	4.7
240	94.1	3.8	102.0	11.6	96.9	5.9

Administration of the control solution of insulin at 2 IU/kg resulted in the mean plasma glucose concentration remaining around the basal levels throughout the study. Nasal administration of the cholylsarcosine and glycodeoxycholate formulations resulted in a mean minimum plasma glucose concentration of approximately 58% (± 5.7 - 6.1).

Example 4

Formulation of Low Molecular Weight Heparin and Cholylsarcosine

A formulation for the administration of low molecular weight heparin to man is prepared as follows:

low molecular weight heparin	174 g
cholylsarcosine	180 g
Avicel®	14 g

The materials are blended in a V-blender and aliquots are filled into starch capsules (obtained from Capsugel AG, Switzerland) using a Bosch filling machine. The formulation is sufficient for the preparation of 1,000 capsules. The mean capsule fill weight is 370 mg.

The capsules are coated with a layer of polymers that will provide release in the colonic region of the gastrointestinal tract, according to the method provided in international patent application No. PCT/GB95/01458.

The method is as follows: Capsules are coated with a solution comprising 20 g of hydroxypropylmethylcellulose (Methocel®; ESM), 2 g of PEG 400 and 200 ml of water. Coating is performed using an Aeromatic STREA-1 fluid bed coater with bottom spray gun. The mean amount of HPMC applied to each capsule is 31 mg.

39 g of Eudragit® L100 and 13 g of Eudragit S100 are dissolved in a mixture of 650 ml of isopropanol and 20 ml of water. 10 g of dibutyl phthalate is mixed into the Eudragit solution. Finally, 10 g of talc is carefully mixed into a paste using 100 ml of isopropanol plasticiser. The coating solution is applied using the Aeromatic STREA-1 fluid bed coater. The capsules coated with HPMC are coated with the Eudragit solution to a mean weight gain of 89 mg per capsule.

The dissolution performance of the capsules coated with HPMC/Eudragit is tested using the USP Method 1 (baskets rotating at 50 rpm). For the first 2 h of the test, 0.1M HCl is used as the test medium. After 2 h, the test medium is changed to 0.05M phosphate buffer, pH 6.8. The dissolution vessels are visually inspected at regular intervals for the appearance of starch residue, which would indicate failure of the coating.

Example 5

Colonic Absorption of a Formulation of Sampatrilat and Cholylsarcosine

A formulation of sampatrilat (Pfizer Ltd., Kent, UK) was prepared by dissolving 1600 mg sampatrilat and 1600 mg cholylsarcosine in 50 ml of ultrapure water, with the pH adjusted to 7 with hydrochloric acid. 6.52 ml of the formulation (32 mg/ml sampatrilat) was administered directly into the terminal ileum, *via* an ileal fistula, in each of 6 pigs. The pigs were surgically prepared as described in Example 1. A simple solution of sampatrilat (32 mg/ml) was administered in the same pigs in a crossover design. Blood was collected in heparinised tubes 15 minutes prior to dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120 and 144 hours after dosing. Plasma was separated by centrifugation and the plasma concentration of sampatrilat measured using an established competitive immunoassay. Results are shown in Table 5.

Table 5. Pharmacokinetic data for the colonic absorption of Sampatrilat in pigs in combination with cholylsarcosine.

Pig Number	Cmax (ng/ml)	Tmax (hours)	AUC (ng/ml.hour)
FORMULATION GROUP 1 (control solution of Sampatrilat)			
1	82.8	0.5	984.2
2	25.0	0.5	778.5
4	126.0	1	1642.9
5	207.0	1	1125.4
6	451.0	0.5	1929.5
7	328.0	0.5	2176.1
Mean (± SD)	203.3 (±160.75)	0.67 (±0.26)	1439.4 (±559.78)
FORMULATION GROUP 2 (solution of Sampatrilat with cholylsarcosine)			
1	17.8	12	1354.6
2	2110.0	0.5	4604.9
3	2460.0	0.5	5043.5
4	2750.0	0.5	5887.3
5	131.0	0.5	1347.4
6	336.0	0.5	1798.7
Mean (±SD) pigs 1-6	1300.8 (±1268.39)	2.4 (± 4.69)	3339.4 (± 2062.93)**
Mean (±SD) pigs 1,5 & 6	161.6 (±161.29)	4.3 (±6.64)	1500.2 (±258.47)
Mean (±SD) pigs 2,3 & 4	2440.0 (±320.47)	0.5 (±0.00)	5178.6 (±651.78)***

It can be seen that in 3 out of 6 pigs the colonic absorption was increased by about 3.6 times for the formulation containing cholylsarcosine as compared to the control formulation.

Claims

1. A composition for administration to a mucosal surface of a mammal comprising a bile salt analogue and a therapeutic agent, characterised in that the bile salt analogue is non-metabolisable.

2. A composition according to Claim 1 wherein the bile salt analogue is a non-naturally occurring conjugate of cholic acid and an amino acid.

3. A composition according to Claim 1 or Claim 2 wherein the bile salt analogue is cholylsarcosine.

4. A composition according to any one of the preceding claims wherein the therapeutic agent is a polar molecule.

5. A composition according to any one of Claims 1 to 4 wherein the therapeutic agent is a peptide, a polypeptide or a protein.

6. A composition according to any one of Claims 1 to 4 wherein the therapeutic agent is a sulphated polysaccharide or glycosaminoglycol.

7. A composition according to Claims 6 wherein the therapeutic agent heparin or low molecular weight heparin.

8. A composition according to any one of Claims 1 to 5 wherein the therapeutic agent is insulin.

9. A composition according to any one of Claims 1 to 5 wherein the therapeutic agent is calcitonin.

10. A composition according to any one of Claims 1 to 5 wherein the therapeutic agent is desmopressin.

11. A composition according to any one of Claims 1 to 5 wherein the therapeutic agent is a growth hormone.

12. A composition according to any one of Claims 1 to 5 wherein the therapeutic agent is a growth hormone releasing factor or growth hormone releasing hormone.

13. A composition according to any one of Claims 1 to 5 wherein the therapeutic agent is parathyroid hormone or a parathyroid related hormone.

14. A composition according to any one of Claims 1 to 5 wherein the therapeutic agent is a colony stimulating factor.

15. A composition according to any one of Claims 1 to 5 wherein the therapeutic agent is erythropoietin.

16. A composition according to any one of Claims 1 to 5 wherein the therapeutic agent is an α-, β- or γ-interferon.

17. A composition according to any one of Claims 1 to 4 wherein the therapeutic agent is an antisense agent such as an oligonucleotide.

18. A composition according to any one of Claims 1 to 4 wherein the therapeutic agent is DNA or a therapeutic gene for gene therapy.

19. A composition according to any one of Claims 1 to 4 wherein the

therapeutic agent is a bisphosphonate.

20. A composition according to any one of Claims 1 to 4 wherein the therapeutic agent is an angiotensin converting enzyme (ACE) inhibitor.

21. A composition according to any one of the preceding claims wherein the mucosal surface is the gastrointestinal tract.

22. A composition according to any one of Claims 1 to 20 wherein the mucosal surface is the buccal cavity.

23. A composition according to any one of Claims 1 to 20 wherein the mucosal surface is the nose.

24. A composition according to any one of Claims 1 to 20 wherein the mucosal surface is the vagina.

25. A composition according to any one of Claims 1 to 21 which is adapted to release therapeutic agent to the small intestine or the colonic region of the gastrointestinal tract.

26. A composition according to Claim 25, wherein the colonic region is the proximal colon.

27. A process for the preparation of a composition according to any one of Claims 1 to 26, which process comprises mixing together a non-metabolisable bile salt analogue and a therapeutic agent in a pharmaceutically acceptable dosing form.

28. A method for the improved administration of therapeutic agents which

comprises administering a composition according to any one of Claims 1 to 26 to a patient.

29. The use of a non-metabolisable bile salt analogue in the manufacture of a medicament for the improved administration of therapeutic agents that are poorly absorbed *via* mucosal surfaces.

30. The use as claimed in Claim 29, wherein the mucosal surface is the gastrointestinal tract.

Figure 1. Plasma Glucose Concentration after Colonic Dosing to Pigs

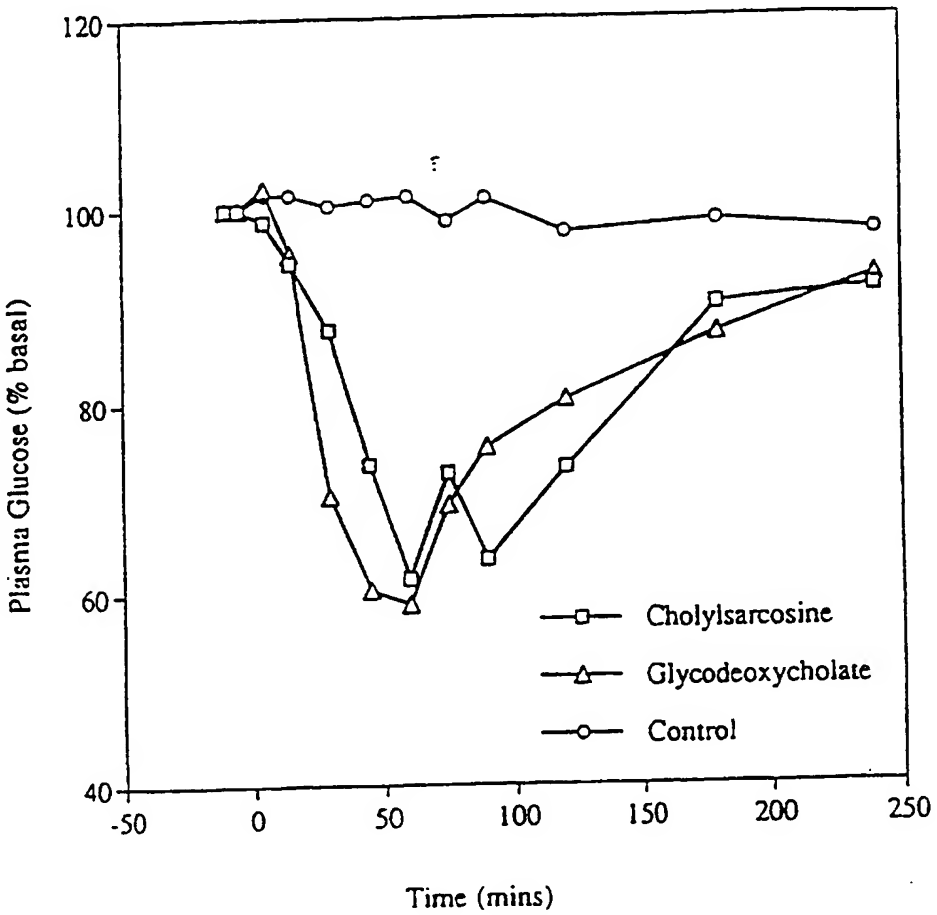

Figure 2. Nasal administration of insulin to sheep with and without enhancer system